GENETIC AND NON-GENETIC APPROACHES TO INCREASING PROLIFICACY IN BEEF CATTLE

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SUMMARY

Productive efficiency of temperate beef cattle herds is presently limited by their relatively low reproduction rate. Attempts to improve reproduction rate have been directed mainly at improving fertility and calf survival. Increasing prolificacy in the cow presents no biological problems and offers greater potential for improving reproduction rate and production efficiency. A progress report on a long term selection experiment for increased prolificacy in cattle is presented and recent developments in non-genetic options for induction of twinning are reviewed.

INTRODUCTION

Productive efficiency of temperate beef cattle herds is presently limited by their relatively low reproductive rate. In NSW herds, for example, each breeding cow weans only 0.8 calves per year (Australian Bureau of Statistics, 1987). Put in another way, each year a cow with a single calf produces about 70% of her liveweight in marketable offspring. By comparison, a sow normally generates about eight times her liveweight each year in weaned pigs. Increasing reproduction rate can increase the efficiency of beef production (Dickerson, 1978) but most attention so far has been directed to improving fertility (proportion of cows pregnant) and calf survival rather than prolificacy (calves born per pregnancy).

Improving prolificacy clearly offers the greatest potential for increasing reproduction rate and the cow is well equipped to produce more than one calf per pregnancy. The bovine ovary is quite capable of producing more than one egg per cycle: hormone treatments to induce multiple ovulations for embryo transfer confirm this. The bovine uterus is also able to carry more than one foetus: embryo transfers confirm this. Finally, the cow has four functional mammary glands and is therefore well endowed to suckle more than one calf. To these attributes must be added the fact that the genes for exceptional milk production are widespread in the cattle population. Given all of this it is surprising that more cows do not have twin births. In most breeds the frequency of twins is less than 2 percent. Yet there are individuals that regularly have twins and some populations within the large European breeds have twinning frequencies as high as 10 percent. Freemartinism should not be regarded as a serious problem since the number of fertile female offspring is not affected by a change in prolificacy and freemartin females have normale growth rates.

In this paper we report progress in a long term selection experiment for increased prolificacy in cattle and review recent developments in non-genetic methods for induction of twinning. Renewed interest in this latter subject has arisen because of recent advances in reproductive technology should markedly improve the reliability of techniques for induced twinning.

GENETIC SELECTION FOR TWINNING

Background

While there are large differences between beef cattle breeds in growth rate and mature size, the differences in litter size are small and for practical purposes insignificant. Because of this, there is an incentive to create strains with increased prolificacy. Renewed interest in genetic manipulation of twinning began in the 1970's with experimental herds selected for increased twinning rate established in France, Australia, the USA and New Zealand. Unlike earlier endeavors (Mechling and Carter, 1964; Stolzenburg and Schonmuth, 1980), these new herds were based on highly selected foundation males and females (Piper and Bindon, 1979). Comparative twinning frequencies for the foundation females before and after purchase and for their first generation daughters were summarised by Morris and Day (1986). Cows with a minimum of two sets of twins prior to purchase averaged around 14 percent of twin births in subsequent calvings, while their daughters averaged around 8 percent of twin births. These frequencies are in general in agreement with expectations based on the repeatability and heritability of twinning and of the approximate selection intensities applied in establishing the respective herds.

CSIRO twinning herd

(i) Foundation animals CSIRO began establishing its twin selection herd in 1973/74 by purchasing cattle with a repeated history of twinning. In all, 65 cows with at least two sets of twins, or a set of triplets or quads prior to purchase, were obtained from herds throughout eastern Australia. Dairy (Jersey, Guernsey, A.I.S., Friesian) and beef (Hereford, Angus, Shorthorn) types were approximately equally represented. The 12 foundation bulls were drawn from a similar array of locations and breeds and were chosen because their dams had unusually high prolificacy (3 to 10 sets of multiple births (10 bulls), or because there was a history of multiple births in the bull's pedigree (2 bulls)). The foundation cows were aged between 2 and 12 years at the time of purchase and 14 of them failed to calve subsequently. For the 51 foundation cows with at least one subsequent calving record, the average prolificacy was 1.11 $\pm$ .05 (123 total records). By contrast, 70 unselected Hereford cows, joined with the twin herd bulls and grazing throughout the year with the selected herd, had an average litter size of 1.01 (234 records).

(ii) Subsequent generations Foundation bulls were joined with foundation cows in November 1974 and 1975 and with foundation cows and their daughters up to and including the joining in November 1980. There was no joining in 1981 due to drought or in 1982 because of a change in the joining time from November to February. All foundation cows and bulls were culled prior to the joining in February 1983.

All females born in the herd up to and including the 1980 drop were retained and given from 6 to 10 opportunities to calve (more for the earlier, less for the later drops). For the 1983 and 1984 joinings, selection of male and female replacements was on the basis of an ad hoc index combining information on the twinning records of the dam and of the maternal and paternal grand-dams. From the 1985 joining until the present, selection of male and female replacements has been based on a formal selection index combining information on the dams' ovulation rate (measured once yearly at joining by mid-ventral laparoscopy) and twinning record, and on the twinning records of the maternal and paternal grand-dams.

Ovulation rate and twinning frequency data for females born from 1975 to 1986 have been included in this paper. The data were analysed using least squares ANOVA procedures according to a model which included effects due to year born (fixed), cow within year born (random) and cow age (fixed). Over all year born groups the mean ovulation rate for a total of 354 cows (904 records) was 1.08 ± .02. In a proportion of cases, a decision could not be made at the time of laparoscopic examination as to whether one or two (or in some cases two or three) corpora lutea were present on the ovary. These records were excluded before analysis. However, if they are added to the data set at the higher of the two possible values, the mean ovulation rate for the same 354 cows was 1.11 ± .02. The mean litter size (344 cows; 998 records) was 1.04 ± .01. This is lower than the value of 1.08 previously reported for first generation daughters of foundation cows and bulls (see Morris and Day, 1986) indicating that mean litter size has declined rather than increased in later year born groups.

(iii) The future The fact that litter size has not increased above the level generated from the initial intense selection of foundation stock is rather disappointing. Genetic options to possibly speed the rate of progress include:

- improved breeding value estimation by making use of BLUP procedures to utilise all information in the pedigree
- M.O.E.T. schemes to make better use of superior females in the CSIRO herd
- introduction of genes from the USDA twin selection herd where the twinning rates in current generation females are around 8 percent (Gregory et al., 1988)
- introduction of genes from high merit bulls progeny tested for twinning in the Scandinavian cattle populations
- introduction of a major gene for twinning if confirmed in New Zealand cattle populations (see Morris and Day, 1986)

However, current difficulties in research funding for long term projects that appear not to be meeting expectations, coupled with the relatively favourable outlook for the non-genetic approaches (see below), may result in an earlier than planned termination of this experiment.

NON-GENETIC METHODS FOR INDUCTION OF TWINNING IN CATTLE

Recent advances in knowlege of reproductive technology have stimulated renewed interest in the non-genetic approach to twinning in cattle. There are three methods under investigation and these are summarized in Table 1.

Twins by hormone injection

The administration of essentially LH-free ovine FSH at known days of the oestrous cycle of adult beef females leads to a modest increase in ovulation rate (Bindon *et al.,* 1986). In that study, oestrous cycles were synchronized by two injections of prostaglandin analogue (PG) at 11-day intervals. On days 8-10 of the subsequent cycle the animals commenced a four-day FSH treatment (two subcutaneous injections per day with ovine FSH of potency 4.5 times NIH-FSH-S_1) with the total dose of 8-10 mg given in a decreasing ratio of 4:3:2:1 over four days. PG was again injected on the morning of the third day. This regime is similar to that used for superovulation in embryo transfer programmes except that the dose of FSH is reduced.

Table 1 Modern methods for inducing twins in beef cattle

Procedure	Recent scientific developments	Potential advantages and disadvantages
Hormone (FSH) injection at known day of cycle	Availability of purified FSH preparations Knowledge of bovine oestrous cycle	Relatively simple technique but expensive and labour intensive. Requires synchronization. Risk of litter size >2. Affects only one cycle
Inhibin vaccine	Immunogens based on native and recombinant inhibins and synthetic inhibin fragments developed in Australia Successful increases in ovulation rate of immunized cattle	Simple procedure. Probably most cost-effective. Does not require synchronization. Therefore, parturition date not predictable. Affects several cycles. Risk of litter size >2.
IVF and embryo transfer	Successful IVF of oocytes from slaughtered cattle Successful culture of embryos *in vitro* to Day 7	Already commercially successful in EEC. Guarantees maximum litter size of 2. Requires synchronization. Parturition predictable. Affects only one cycle. May not be cost-effective

An example of the results is shown in Table 2. Although the mean ovulation rate is close to the desired level, about half the treated animals have a single ovulation and some have ovulation rates outside the desired range (say 2-4).

With present knowledge it is difficult to see how the precision of this technique could be improved. Simply increasing the dose of FSH will not help because a larger proportion of the population will then have ovulation rates in the undesirable high range. Still the results in Table 2 (i.e., with about 50% of cows with 2-4) are better than expected from treatments with, say PMSG, and may be acceptable if they could be reproduced on a large scale.

The bovine response to exogenous FSH is dependent on the age and/or liveweight of the animals. For example, in a treatment protocol as described for Table 2, Hereford heifers aged 15 months and weighing 260 kg produce a mean of 9.50 ovulations (range 1-14) in response to 8.0 mg ovine FSH. Mature Herefords weighing 450 kg produce 2.92 ovulations (range 1-7) in response to the same dose (B.M. Bindon and L.R. Piper, unpublished observations).

Table 2 Distribution of ovulation rates in three-year-old nulliparous Hereford females treated with low dose ovine FSH (B.M. Bindon and L.R. Piper, unpublished)

Treatment	n	Number of cows with ovulation rates of: 1	2	3	4	5-10	>10	Mean ±SE
Nil	18	18	-	-	-	-	-	1.00±0
Ovine FSH (8-10 mg over 4 days)	38	17	8	5	3	4	1	2.61±0.42

If cattle of mixed age are treated with a fixed dose of 8.0 mg ovine FSH an ovulation rate distribution, conception rate and twinning rate as in Table 3 are observed. Note that these are synchronized females naturally mated only at the synchronized oestrus. It is clear that with ovulation rate in the range 2-4, fertility is comparable to that of single ovulators and 27% of treated females produce multiple births. When ovulation rates exceeded four, fertility was poor and few cases of twins resulted.

Table 3 FSH-induced twin pregnancies in synchronized, mixed age beef females treated with 8.0 mg ovine FSH (B.M. Bindon and L.R. Piper, unpublished)

Number of ovulations	No of cows	% of herd	Cows calving No	Cows calving %	No with litter size: 1	2	3	% multiple births Cows treated	% multiple births Cows calved
1	24	22	11	46	11	0	0	0	0
2-4	41	37	18	44	7	8	3	27	61
5-8	21	19	2	10	0	2	0	10	100
9-20	23	21	3	13	1	2	0	9	66
Total	**109**		**34**	**31**	**19**	**12**	**3**	**14**	**44**

So in this experiment with poor control of ovulation rate, from 109 animals treated, 34 calved and 15 produced multiples (12 sets twins, 3 sets triplets). If it can be assumed that the 75 cows which did not conceive (i.e., in Table 3) to the treatment cycle would have normal fertility at the next cycle (say 0.9 calves born per cow joined), then the following outcome would apply:

Cows joined	=	109
Cows calved	=	(34 + 68)....102
Single calves born	=	(19 + 68)......87
Twin calves born	=	(12 x 2)........24
Triplet calves born	=	(3 x 3)...........9
TOTAL CALVES BORN	=	120
∴ Calves born/cow joined	=	1.10

It is unlikely that FSH treatments as described here would produce economically useful increases in productivity even if all extra calves survived. The cost of synchronization, labour and FSH treatment would amount to at least $A20 per cow treated, so the extra calves (20 per 100 cows joined if the "normal" figure is 90 calves per 100 cows joined) would need to be worth $A100 each just to break even. Until a more reliable response to FSH injection can be assured the technique will remain a research tool.

Twins by inhibin vaccine

This approach deals with a vaccine against the protein hormone, inhibin, which is a natural peptide produced by the ovary and which acts on the pituitary to keep FSH secretion under control. As shown in Table 4, preliminary CSIRO experiments (Bindon *et al.*, 1988) have been successful in increasing ovulation rate in commercial cattle by vaccination with two forms of inhibin. Ovulation rates remain elevated for more than one oestrous cycle. Further work work will involve evaluating the most appropriate vaccine from a range of inhibins produced using recombinant DNA techniques by Biotechnology Australia. Vaccination has the advantage of simplicity and economy as well as ease of implementation. It will not be necessary to know the stage of the cow's cycle, so synchronization will not be needed. The potential disadvantages are the risk of triplets and the fact that day of parturition will not be predictable (since day of conception will not be recorded). Precise control of ovulation rate may not be essential since the data in Table 3 indicate that normal fertility and acceptable twinning frequencies result when ovulation rate does not exceed 4.

Table 4 Increased ovulation rates in commercial cattle vaccinated against inhibin preparations (B.M. Bindon, L.R. Piper and T. O'Shea, unpublished)

Preparation	Cattle	n	Group	Number of ovulatory cycles examined	Percentage with >1 ovulation	Mean number of ovulations (±SE)
Native ovine inhibin	Heifers (250 kg)	8	Control	21	0	1.0 ± 0
		8	Immun.	25	80	8.7 ± 1.9
Synthetic fragment	Cows (600 kg)	8	Control	22	0	1.0 ± 0
		8	Immun.	23	22	1.3 ± 0.11

Twins by embryo transfer

The third technique involves the transfer of two foreign embryos to an unmated cow (ET twins) or one supplemental embryo to a cow already joined (SET). The transfers are carried out on Day 7. Although these procedures are not new, they have become more attractive due to recent advances in *in vitro* fertilization (IVF) and embryo culture. As shown in Table 5 bovine oocytes from slaughtered cattle can be fertilized and cultured through to Day 7 on a bed of cells from the oviduct. This means that large banks of frozen embryos of known breed can be made available for ET twinning without the great expense that is incurred when embryos are collected from live donors treated with hormones.

Table 5 Fertilization *in vitro* and culture of cow eggs on a bed of tubal epithelial cells (from Thibault and Crozet, 1988)

		Development to:		
Length of capacitation	Fertilization (%)	Four cell (%)	Morula (%)	Blastocyst (%)
Short (90 min)	80	40	66	47
Long (4 hour)	92	46	31	25

This break-through has revolutionized the ET approach to induced twinning. It has already reached commercial application in Ireland and parts of the United Kingdom (see Lu *et al.*, 1987). New methods for sexing embryos (Matthews *et al.*, 1987) can be expoited so that twins of known breed and sex can be guaranteed. ET is being used in Ireland to produce 3/4 continental breed calves from the Friesian dairy herds. This technique has the advantage that litter size is fixed at a maximum of two and date of parturition can be predicted. Disadvantages are the potential cost of embryos, synchronization and transfer.

CSIRO is collaborating with the NSW and Victorian Departments of Agriculture and other institutions to evaluate this approach in a co-ordinated project funded by AMLRDC. The idea is to develop appropriate management strategies at Grafton (NSW) and Hamilton (Victoria) for beef females (Hereford/Angus cross) carrying twins . This is being done on the assumption that either the inhibin vaccine approach or the IVF/ET twinning method will be available commercially in Australia by 1992.

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